identify sequences having about 80% or more sequence identity with the probe.

Moderate and high stringency hybridization conditions are well known in the art (see, for example, Sambrook, *et al,* 1989, Chapters 9 and 11, and in Ausubel, F.M., *et al.*, 1993, expressly incorporated by reference herein). An example of high stringency conditions includes hybridization at about 42°C in 50% formamide, 5X SSC, 5X Denhardt's solution, 0.5% SDS and 100 μg/ml denatured carrier DNA followed by washing two times in 2X SSC and 0.5% SDS at room temperature and two additional times in 0.1X SSC and 0.5% SDS at 42°C.

As used herein, "recombinant" includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid sequence or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found in identical form within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all as a result of deliberate human intervention.

As used herein, the terms "transformed", "stably transformed" or "transgenic" with reference to a cell means the cell has a non-native (heterologous) nucleic acid sequence integrated into its genome or as an episomal plasmid that is maintained through two or more generations.

As used herein, the term "expression" refers to the process by which a polypeptide is produced based on the nucleic acid sequence of a gene. The process includes both transcription and translation.

The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means "transfection", or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid sequence into a eukaryotic or prokaryotic cell where the nucleic acid sequence may be incorporated into the genome of the cell (for example, chromosome, plasmid, plastid, or mitochondrial DNA), converted into an

autonomous replicon, or transiently expressed (for example, transfected mRNA).

Proteolysis in bacteria serves to rid the cell of abnormal and misfolded proteins. A unique mechanism for the destruction of abnormal proteins resulting from abortive termination of translation is provided by the SsrA-mediated tagging and degradation system (for a recent review, see Karzai et al. 2000. The SsrA-SmpB system for protein tagging, directed degradation and ribosome rescue. Nat. Struct. Biol. 7:449-455). SsrA, also called 10Sa RNA or tmRNA is a highly conserved RNA molecule in eubacteria. It is a unique molecule that can act as both a tRNA and an mRNA in a process referred to as *trans*-translation (Atkins et al. 1996. A case for *trans* translation. Nature 379:769-771; Jentsch 1996. When proteins receive deadly messages at birth. Science 271:955-956; Keiler et al. 1996. Role of a peptide tagging system in degradation of proteins synthesized from damaged messenger RNA. Science 271:990-993). This mechanism provides the cell a way to release ribosomes that are stalled on untranslatable mRNAs, e.g. mRNAs lacking in-frame stop codons. In the model for SsrA action, SsrA charged with alanine enters the A site of a stalled ribosome, mimicking a tRNA. The alanine is added to the uncompleted polypeptide chain; and then, serving as an mRNA, SsrA provides a short reading frame followed by a stop codon as a template to add a short peptide to the nascent polypeptide before translation terminates and a tagged protein is released. The peptide tag (encoded by SsrA) functions as a proteolytic degradation signal, and in *Escherichia coli* four proteases have been identified that degrade proteins tagged by SsrA. ClpXP, ClpAP, and FtsH (HflB) degrade SsrA tagged proteins in the cytoplasm (Gottesman et al. 1998. The ClpXP and ClpAP proteases degrade proteins with carboxy-terminal peptide tails added by the SsrA-tagging system. Genes Dev. 12:1338-1347; Herman et al. 1998. Degradation of carboxy-terminal-tagged cytoplasmic proteins by the *Escherichia coli* protease HflB (FtsH). Genes Dev. 12:1348-1355), while SsrA tagged proteins with

signal peptides that are exported to the periplasm of *E. coli* are degraded by Tsp (Prc) protease (Keiler et al. 1996).

Not only ribosome stalling on messages without in-frame stop codons leads to activation of the SsrA tagging system. It also occurs when ribosomes stall at clusters of rare codons in an mRNA when the cognate tRNA is scarce (Roche et al. 1999. SsrA-mediated peptide tagging caused by rare codons and tRNA scarcity. EMBO J. 18:4579-4589), and there may be more conditions that result in SsrA tagging. The whole story leading to the elucidation of SsrA function started with the observation by Tu *et al.* (1995. C-terminal extension of truncated recombinant proteins in *Escherichia coli* with a 10Sa RNA decapeptide. J. Biol. Chem. 270:9322-9326) that a fraction of mouse interleukin-6 expressed in *E. coli* is truncated and contained the SsrA tag. It is not clear why in this case part of the mIL-6 molecules were tagged by SsrA. Perhaps mIL-6 mRNA is relatively unstable in *E. coli*, leading to transcripts that are trimmed at the 3' end by nucleases, thereby losing its stop codon. Alternatively, mIL-6 overexpression itself may lead to jamming at the ribosomes, thereby activating the SsrA tagging system. Whatever the reason is, contamination of recombinant proteins with molecules that are truncated and tagged by the SsrA system (and escape from degradation) restricts the usefulness of these molecules e.g. as pharmaceutical proteins. Therefore, peptide tagging according to the present invention, i.e., the utilization of a charged tag, in *B. subtilis,* an industrially important species used for the commercial production of various proteins provides a substantial benefit not found in the prior art.

B. subtilis SsrA has been isolated and sequenced several years ago (Ushida et al. 1994 tRNA-like structures in 10Sa RNAs of *Mycoplasma capricolum* and *Bacillus subtilis*. Nucleic Acids Res. 22:3392-3396) and the sequence of the proteolysis tag encoded by *B. subtilis* SsrA ((A)GKTNSFNQNVALAA) has been predicted (Williams 2000. The tmRNA website. Nucleic Acids Res. 27:165-166). Recently, Wiegert and Schumann